

SCREENING PLANT GROWTH REGULATORS FOR MODIFICATION OF
HOST PLANT RESISTANCE TO MELOIDOGYNE INCOGNITA

by

Thomas Vern Orum

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SIGNED:

Thomas V. Quinn

APPROVAL BY THESIS DIRECTOR

This thesis has been approved on the date shown below:

Paul G. Bartels

P. G. BARTELS

Associate Professor of Plant Sciences

Aug 12, 1977
Date

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ABSTRACT

Tomato plants susceptible to root knot nematode were treated with plant growth regulators by soil drench, inoculated with 2,000 nematode larvae/pot one week later, and harvested four to six weeks after inoculation. Roots were evaluated by knot counts, gall index, and egg counts.

Tomato plants treated with 10 to 15 ppm oryzalin showed more than 90% reduction in knot counts. Oryzalin did not kill nematodes on direct contact; therefore, its effect was through the plant. Oryzalin affected penetration, but treatment one week after inoculation reduced knot counts and indicated oryzalin also affected gall formation after nematodes had penetrated. Plants 6 cm tall or less at time of treatment were severely damaged by oryzalin. Treatment was most successful when applied to plants 12 cm or taller.

Tomato plants treated with 10,000 ppm 1,1-dimethylpiperidinium chloride (BAS 083 00W) showed reductions in knot counts and egg counts. That BAS 083 00W and oryzalin were able to reduce nematode activity without severe root pruning suggested that modification of plant resistance to nematodes with plant growth regulators was feasible. High correlation of egg counts with knot counts indicated that

counting eggs could be used in screening plant growth regulators that increase tomato plant resistance to nematodes.

INTRODUCTION

Modification of tomato plant resistance to root knot nematode using plant growth regulators was selected to test the more general concept of pest control by chemically induced changes in the plant. The inspiration for the project came from the observation that plant resistance to disease and insects is a widely occurring phenomenon and that this resistance is most likely due to special biochemical and morphological features of the plant pest interaction. There are a wide variety of plant growth regulators and herbicides which modify plant biochemistry and morphology. If known plant active substances show promise in a pest control system, it might prove worthwhile for an industrial firm screening chemicals for plant growth regulator effects to include tests for modification of host plant resistance in their screening program. The goals of the research effort with root knot nematode and tomato plants therefore were to provide a case study: (1) to test the feasibility of increasing plant resistance to pests using plant growth regulators and (2) to suggest techniques by which large numbers of compounds might be screened for such activity.

REVIEW OF THE LITERATURE

This review is divided into four sections. The first section is an historical overview of reports that plant growth regulators and herbicides modify host plant resistance to disease and insects. The second section summarizes reports of plant growth regulators modifying resistance to nematodes. The third section reviews suggestions on how such chemicals might modify resistance to nematodes. The fourth section discusses screening techniques in systemic nematocidal research.

History of Chemical Modification of Host Plant Resistance

The concept that plant growth regulators can modify host plant resistance to pests developed in three ways:

(1) as new chemicals became available they were tested on host-parasite systems being studied for other reasons, (2) the research for systemic pesticides often led to plant growth regulators, (3) herbicide-pathogen interaction studies indicated compounds which changed host plant resistance.

Modifying plant resistance with plant growth regulators is an idea that is about thirty years old. During and immediately after World War II, there was intense research for biologically active compounds spurred by the

popular success of DDT in the war effort. The newly synthesized chemicals were applied to practically every biological system then being studied. The insecticide DDT was reported to affect plant growth (Allen 1947). Plant growth regulators and herbicides were reported to affect a wide variety of fungi (Richards 1949, Rowell 1949, Hsia and Christensen 1951) and viruses (Hartman and Price 1950, Kutsky and Rawlins 1950). Allen (1947) suggested that a chemical could protect a plant from pests by acting on the plant rather than on the pest.

As new plant growth regulators became readily available over the years, they were tested on a wide variety of systems: bacteria (Crossan and Fieldhouse 1964), cucumber scab (van Andel 1968), Dutch Elm Disease (Beckman 1958), virus (Kiryaly and Szirmai 1964), gall mites (Smith and Corke 1966), aphids (van Emden 1964), and nematodes (Nusbaum 1958). Often the reported plant growth regulator effects were not central to the work of the researcher and were not pursued.

In 1953, Davis and Dimond of the Connecticut Agricultural Station wrote an article entitled "Inducing disease resistance with plant growth regulators." They screened systemic fungicides for Fusarium wilt on tomato and discovered that some of their compounds were active not because they were fungitoxic, but because they changed the plant in some way. In the following twenty years, Dimond

tested over 250 compounds to find those which reduced Fusarium wilt on tomatoes without being toxic to the fungus itself (Davis and Dimond 1953, Corden and Dimond 1959, Biehn and Dimond 1973). Twelve compounds at appropriate concentrations satisfied their criteria including 2,4-dichlorophenoxy acetic acid (2,4-D), α -naphthalene acetic acid (NAA), 2,3,5-tri-iodobenzoic acid (TIBA), indole-3-acetic acid (IAA) (Davis and Dimond 1953), and ethrel (ethepon) (Biehn and Dimond 1973).

Another researcher who studied both systemic fungicides and plant growth regulators in disease control is D. D. Erwin (Erwin, Tsai, and Khan 1976; Buchenauer and Erwin 1976). Erwin reported that the new growth retardants pydanon and tributyl ((5-chloro-2-thienyl-methyl) phosphonium chloride reduced verticillium wilt disease of cotton in field tests. Field successes, such as Erwin has reported, are rare in the literature.

The third source of reports of host plant resistance modification by chemicals is found in studies of herbicide-plant pathogen interaction. Katan and Eshel (1973) have written an excellent review of these studies. The authors point out that plant disease is the result of a complex chain of events involving host plant, pathogen, and surrounding micro-flora and fauna growing in a micro-environment. Most chemicals affect more than the target organism; therefore, the possibilities for a chemical to

have a profound indirect effect on the system is large and the complexity of the interactions make causes and effects difficult to determine.

Largely absent from the history of modification of host plant resistance by using plant growth regulators is the group of people who have worked most closely with host plant resistance in the field--the plant breeders and collaborating entomologists and pathologists. Traditionally, plant breeders do not consider a plant resistant unless the property is heritable. The term "expression of resistance" is used to describe the fact that in various stages of growth and under various environmental conditions a particular variety or genotype will exhibit more or less damage in the field. In this terminology, the goal of using growth regulators is to modify the expression of resistance--a goal often subordinate to finding genetic resistance.

Modification of Resistance to Nematodes

Nusbaum (1958) reported that foliar application of maleic hydrazide (MH) to tobacco plants at levels of 10 mg to 25 mg/plant reduced nematodes, although at these levels the plants were damaged. Peacock (1959, 1960, 1963) studied chemically induced resistance to root knot nematode. He confirmed the effect of MH and its problem of phytotoxicity. Feldman and Hanks (1963) attempted to control

the burrowing nematode on grapefruit seedlings in the field but were not successful. Banks (1970) saw no effect with treatment of 1 mg MH per peanut plant infected with Meloidogyne hapla. Mjuge and Viglierchio (1975) reported a reduction in the number of knots on tomato plants treated with MH. Davide and Triantaphyllou (1968) studied the effect of foliar application of maleic hydrazide on the sex differentiation of M. javanica on tobacco and tomato plants. They found that plants treated with MH following inoculation had a much higher incidence of males than untreated control plants. Plants treated with MH prior to inoculation were resistant to penetration. They suggested the change in sex ratio was due to suppression of giant cell formation and the effect on penetration was due either to suppression of attractants at the root tip or to altered tissue development at the root tip.

Kochba and Samish (1971, 1972) studied the effect of nine selected plant growth regulators on varieties of peach seedlings resistant and susceptible to Meloidogyne javanica. The chemicals tested were NAA, kinetin, MH, thiourea (TU), actidone (AD), TIBA, 7-aza-indole (ACI), 2-hydroxy-5-nitro-benzyl bromide (HNB), and 2,6 diamino purine (DAP). They measured the effect on galling using a five point gall index and the effect on nematode development and reproduction by counting adult females and egg masses. At concentrations in the range of 1 ppm, NAA and

kinetin separately and together synergistically destroyed resistance in resistant peach varieties (Kochba and Samish 1971). MH, TU, TIBA, and at high concentrations AD inhibited gall formation and reduced the number of adult females and egg masses on susceptible plants. At lower concentrations, AD inhibited the number of adult females and egg masses, but not the number of galls. They found that AZI, HNB, and DAP were not very effective. None of the chemicals damaged the plants at the concentrations used (Kochba and Samish 1972). Dropkin, Helgeson, and Upper (1969) reported a loss of resistance in tomatoes when kinetin was applied, an effect similar to that observed by Kochba and Samish. Sawhney and Webster (1975) reported that .05 ppm NAA and 1.07 ppm kinetin stimulated galling in resistant tomato plants without stimulating larval development. They concluded resistance was not completely broken in their case.

Orion and Minz (1969, 1971) have studied the effect of ethrel and morphactin on Meloidogyne javanica infected tomato plants. They found that in dosages from 1 mg to 20 mg ethrel per plant, gall weight increased although the amount of galling per se was not affected. Sample galls were examined for differences in number or size of giant cells. No differences were found. The authors suggested that the effect of ethrel was on proliferation of the parenchymal tissue that surrounds the giant cells (Orion

and Minz 1969). On the other hand, they found that morphactin suppressed giant cell formation and altered nematode development. Adult females appeared distorted and few females in treated plants were capable of egg laying.

In addition to the plant growth regulator work, there has been considerable research done on nematode-herbicide interaction. Authors reporting on this include Chappell and Miller (1967); Altman and Ross (1967); Webster (1967); Anderson and Griffin (1972); Romney, Anderson, and Griffin (1974); and Johnson, Dowler, and Hauser (1975). Romney et al. (1974) reported increased resistance to M. hapla in onion seedlings treated with demethyl tetra-chloroterephthalate (DCPA). Knot counts were made four weeks after inoculation. Control plants had knot counts greater than 25; treated plants had knot counts less than 5. Plant height was unaffected by treatment. Root weight showed a trend to be lower in treated plants inoculated with nematodes. Opposite to their result with DCPA, Anderson and Griffin (1972) found increased nematode damage on plants treated with trifluralin.

Romney et al. (1974) presented two pieces of evidence that the effect of DCPA was on plant resistance rather than on the nematode directly. First, larvae incubated in DCPA for 24 hours prior to inoculation infected onion plants as well as untreated larvae did. Second, plants grown in treated soil and transplanted to untreated

soil at the time of inoculation showed fewer knots than controls. Anatomical studies showed that the larvae that succeeded in penetrating DCPA treated plants developed normally. They concluded the increased resistance was due to the plant's injury response to the herbicide creating either a mechanical or chemical barrier to nematode penetration.

As occurred in the study of systemic fungicides, the study of systemic nematicides led to plant growth regulators. Nelmes, Trudgill, and Corbett (1973) have reviewed an extensive literature on nematode control by chemotherapy in which both systemic nematicides and plant growth regulators were discussed. Peacock (1959, 1960, 1963, 1966, 1968) studied foliar sprays of plant growth regulators such as MH and systemic nematicides such as thionazin and aldicarb.

Possible Explanations for Modifications in Nematode Resistance

An understanding of the biology of the root knot nematode is necessary to theorize on how plant growth regulators might affect resistance. Dropkin et al. (1969, p. 55) summarized the important points:

Larvae of Meloidogyne enter a host at or near the root tip and migrate intercellularly to the region of differentiating vascular tissue. They become sedentary and, within a susceptible plant, the larvae begin to grow while the surrounding root tissues undergo redifferentiation. Root cells near a nematode's head display hypertrophy with

repeated nuclear divisions, together with incorporation of neighboring cells; this results in large, thick-walled, multi-nucleate syncytia ("giant cells"). . . . Pericycle cells divide and enlarge to form galls; xylary differentiation is disrupted. Lateral roots frequently grow from the galls. In resistant plants, however, this pattern does not develop. Larvae may enter roots in low numbers, or the syncytia may develop abnormally. In the most common resistant reaction, larvae enter the roots but the cells immediately surrounding the larvae die, entombing them. The nematodes presumably starve.

Peacock (1959) suggested several ways in which a chemical might modify plant resistance to nematodes: (1) by masking the attractant substance in the root, (2) by killing the nematode, (3) by neutralizing the effect of the nematode on giant cell development, (4) by changing cell wall composition so chemicals secreted by the nematode can not enter the cell or that the nematode stylet can not penetrate it, or (5) by upsetting the sex-ratio in the nematode population.

Plant growth regulators can affect enzyme activity. Veech and Endo (1970) reported differences in enzyme activity at the site of the nematode in soybeans susceptible and resistant to root knot nematode. A similar effect might be induced by a plant growth regulator to help contribute to resistance. Another possibility for chemical modification is suggested by a morphological character associated with resistant soybean varieties. Crittenden (1954) reported that a root system with long tapering roots that possessed a minimum of lateral roots

appeared to be associated with root knot resistance in soybeans.

Reilly and Klarma (1972) suggested another method by which chemicals might induce disease resistance--the stimulation of phytoalexin production. Phytoalexins are compounds produced by the plant in response to the pathogen and which are toxic to the pathogen (Kuc 1966). In soybeans the phytoalexin is hydroxyphaseolin (HP). Reilly and Klarma (1972) noted that many fungicides were more toxic in vivo than the in vitro tests suggested that they should be. Testing twenty-seven fungicides, they found fifteen that increased HP production in soybean hypocotyls. The common denominator among these compounds was that they were either amines or released amines on decomposition. The authors suggested that short chain amines might aid in disease control by inducing the plant to produce phytoalexins.

The phenomenon to be explained by the above suggestions is that the signs and symptoms of nematodes are reduced by chemicals which do not kill the nematodes on direct contact. All suggestions so far involved plant modification. Another possibility is that the chemical does not kill the nematode but alters its behavior in the presence of the plant. Two systemic nematicides, thionazin and aldicarb, do not kill nematodes but rather affect their stylet function preventing penetration (Nelmes et al.

1973). This subtle effect on the nematode would be difficult to distinguish experimentally from an effect due to plant modification.

Screening Techniques in Systemic Nematicide Research

Bunt (1975) estimated that chemical companies screen over 100,000 compounds for nematicide activity each year. The common screening process consists of three stages: (1) a primary screen, (2) a secondary test, and (3) field tests. The goal of a primary screen is to check many treatments using as little space, labor, and time as possible. The secondary test is to verify the effect of those compounds discovered in the primary screen. The field test is used only for the most promising compounds.

Four primary screening methods have been used in the past: (1) the water screen, (2) the soil screen, (3) the Pisum test, and (4) the biological assay with root knot nematode (Bunt 1975). The water screen is simply incubating the nematodes for 1 or 2 days in the chemical solution and observing mortality. In the soil screen, nematodes are incubated in treated soil, washed free of the soil, and observed for mortality. The Pisum test is designed specifically for systemic nematicides which may have little killing effect on the nematodes in the first two screens. The Pisum test uses the nematode Ditylenchus dipsaci on germinating peas that have been soaked in the

chemical. Evaluation takes place two weeks after germination. In the biological assays with root knot nematodes, tomatoes are grown in treated and inoculated soil. The roots are evaluated by gall index four to six weeks later (Bunt 1975).

Culturing excised tomato root tips was investigated by Peacock (1959, 1960) and Dropkin and Boone (1966) as a possible primary screen for systemic nematicides. Peacock was not successful in using the cultured roots to indicate systemic nematicides and plant growth regulators which are effective on whole plant systems (Peacock 1960). Dropkin and Boone (1966) were more optimistic. They set up a system similar to Peacock's which they said can be handled by one person to test 400 units weekly. Unlike Peacock, Dropkin and Boone did not test their system with plant growth regulators. They used mineral elements and plant root extracts.

Three new primary screens were tested by Bunt (1975). All involved evaluation of stem cuttings rooted in 5 ml of dry silver sand in 10 ml glass vials. These systems allowed for in vivo testing of large numbers of compounds in small spaces. The first procedure used bean stems (Vicia faba) inoculated with D. dipsaci and evaluated for penetration after 24 hours. The second test used tomato stems (Lycopersicum esculentum) inoculated with D. dipsaci and evaluated for stem swelling after 6 to 8 days.

The third test used tomato stems rooted for a few days, inoculated with M. incognita, and evaluated by gall index after 7 to 10 days. Using two replicates of three concentrations per chemical, it was possible to test about 250 chemicals per person per week with each of these methods (Bunt 1975).

MATERIALS AND METHODS

Materials

The root knot nematodes, Meloidogyne incognita Chitwood, which were used in these experiments, originated from a single egg mass and were maintained on chile pepper plants, Capsicum frutescens. Susceptible tomato varieties Bonnie Best and Floradel (Lycopersicum esculentum) were used as test plants. Table 1 lists plant growth regulators and herbicides tested. The common name or experimental number is used in the text. The potting soil was a mixture of local topsoil with twenty mesh silica sand in the proportion 2 gal topsoil to 100 pounds sand.

Standard Techniques

Growing the Tomato Plants

Tomato seeds were planted in vermiculite in 15 cm pots and after four to seven weeks of growth, individual seedlings were transplanted into 10 cm pots containing the potting soil. In some experiments, two seeds were germinated directly in potting soil of 10 cm pots. After emergence, seedlings were thinned to one plant per pot and 15 of the most uniform plants were selected for use.

Table 1. Plant growth regulators tested in the research.

Common name or experimental number	Chemical name	Source
Abscisic Acid	(R-(Z;E)-5-(1-hydroxy-2,6,6-trimethyl-4-oxo-2-cyclohexen-1-yl)-3-methyl-2,4-pentadienoic acid	Sigma Chemical Company
Ancymidol (A-Rest)	alpha,-cyclopropyl-alpha-(4-methoxyphenyl)-5-pyrimidinemethanol	Elanco
BAPN	beta-amino-propionitrile fumarate	Sigma Chemical
BAS 083 OOW	1,1-dimethyl-piperidinium chloride	BASF-Wyandotte
Cytex	natural blend of cytokinins 100 ppm cytokinin activ.	Atlantic and Pacific Research Inc.
EPTC	S-ethyl dipropyl-thiocarbamate	Stauffer Chemical Company
Ethrel	(2-chloroethyl) phosphonic acid	Amchem
Gibberellic Acid (GA)	2,4a,7-trihydroxy-1-methyl-8-methylene gibb-3-ene-1, 10-carboxytic acid-1-4-lactone	Calbiochem
Glyphosine	N,N/bis (phosphonmethyl) glycine	Monsanto
IAA	indole-3-acetic acid	Calbiochem
Kinetin	6-furfurylamino-purine	Nutritional Biochemicals
Maleic Hydrazide (MH)	1,2-dihydro-3,6-pyridazinedione	Uniroyal

Table 1.--Continued

Common name or experimental number	Chemical name	Source
NAA	alpha-naphthalene acetic acid	Amchem
Oryzalin	3,5-dinitro-N ⁴ ,N ⁴ - dipropyl-sulfanilamide	Elanco
Phosphon	tributyl-2,4-dichloro- benzyl phosphonium chloride	Mobil
Virazole	1-beta-D-Ribofuranosyl- 1,2,4-triazole-3- carboxamide	ICN Pharmaceuti- cals

Methods of Treatment, Inoculation, and Harvest

Chemicals were dissolved in either water or 4-10% acetone and applied to plants as a soil drench (15 ml per plant) or as a foliar dip. No surfactants were used unless present in the formulation. Soil moisture was usually just below field capacity at time of treatment. For the foliar dips, the soil was covered with aluminum foil and the plant inverted into a beaker containing the solution for 15 seconds. The foil remained on the soil until the solution dried on the plant and prevented the chemical from dripping onto the soil. Plants were treated from three to five weeks after transplanting and were inoculated one week after treatment.

Plants were inoculated with second stage nematode larvae within 48 hours of hatching. Nematode eggs were obtained from the roots of the chile pepper plant by shaking the roots in 20% household bleach, screening the eggs through a 100 mesh sieve and collecting them in 400 and 500 mesh sieves. The eggs were surface sterilized for twenty minutes with Hibitane, rinsed with water, and placed in a water bath to hatch over a fourteen-day period. Collected larvae were diluted to a concentration of 100 larvae per ml with distilled water. Twenty ml of larvae suspension were applied directly to the soil in each pot.

Precautions were taken so that larvae were not exposed to ultra violet light.

Roots were harvested and plant heights measured four to seven weeks after inoculation. The roots were collected by cutting the stem and gently washing the soil away in a bucket of water. The roots were then wrapped in a paper towel, placed in a labeled plastic bag, and refrigerated until evaluated.

Methods of Evaluating Roots

The effect of the plant growth regulators and the nematodes on the roots was evaluated in four ways. All roots were evaluated using knot counts and dry root weight. In addition, many roots were evaluated using the gall index and egg counts.

Knot Counts. Roots were placed in a petri dish, covered with water, and carefully spread apart using dissecting needles. The dish was placed over a grid made with 1 cm wide red tape on a gray cardboard background. Knots were visible to the unaided eye, but were counted more easily under a 2x magnifying lamp.

Gall Index. The gall index is an estimate of the per cent of the roots that have knots on them. Roots were rated subjectively from 0 to 10 based on the degree of galling. To display the roots for rating, the roots were

floated in a beaker of water and illuminated with fluorescent light from the side of the beaker.

Egg Counts. Egg counts were done prior to knot counts and as soon after harvesting the roots as possible, usually within 24 hours. All five roots of a given treatment were marked for identification and combined for egg count analysis. The volume of the set of roots was estimated by water displacement in a beaker. The roots were placed in a flask to which 20% household bleach was added in an amount roughly 4 times the estimated root volume. This was usually between 100 ml and 400 ml. The roots were swirled for 3 min in the bleach to dislodge the eggs. The resulting suspension was poured through a 60 mesh screen cup nested in a 500 mesh screen cup where the eggs were collected. The eggs were resuspended in 500 ml of water and allowed to stand exactly 2 min to allow dirt particles to settle. At 2 min the upper 100 ml was poured into a small beaker. Three 1 ml samples were taken from this 100 ml and counted on a Peter's counting slide. Six of the twenty-four squares on the slide were counted and the counts for these three samples averaged.

Dry Root Weights. After other evaluations were completed, roots were trimmed of remaining stem, dried at 60 C for 24 to 48 h and weighed to the nearest .01 gm.

Experimental Designs

Completely Randomized Block Design

This design was used to evaluate many of the compounds for the first time. The only variable in these experiments was the type of treatment. The number of treatments, n , varied from 3 to 9. The number of replications for each treatment, m , was either 4 or 5. The m times n plants were arranged randomly in trays on the greenhouse bench. Data were analyzed using Dunnett's procedure for comparing treatment means with control means (Steel and Torrie 1960). The square root transformation was used for knot count data because these data were distributed according to the Poisson distribution.

Split Plot Design

The split plot design (Little and Hills 1972) was used for two factor experiments where treatment and plant age at time of treatment were varied. Main plots were three different age groups (4, 6, and 8 week old plants at time of treatment). The main plots were arranged according to a randomized complete block design to determine if position of the pots on the greenhouse bench influenced the data. Three plants of each age group were assigned to a block. There were five blocks of nine plants per block arranged east to west on the greenhouse bench. Within each

block the three plants of each age group received different treatments (two chemical treatments and an untreated control). Knot count data were transformed by square root. Duncan's Multiple Range Test was used for mean separation of treatments within age groups (Little and Hills 1972).

Secondary Tests of Oryzalin and BAS 083 00W

The following experiments were designed to investigate in more detail the effects of treatment with oryzalin and BAS 083 00W.

Varying the Time Between Treatment and Inoculation

In this experiment the same amount of the chemical was applied to four sets of five plants in turn two weeks before inoculation, one week before inoculation, at the time of inoculation, and one week after inoculation. Oryzalin was applied as a soil drench at the rate of 200 $\mu\text{g}/\text{pot}$ and BAS 083 00W was applied as a soil drench at the rate of 138 mg/pot . A set of five untreated plants was included as controls. The plants were six weeks old at the time of inoculation. The randomized complete block design was used, and data were analyzed using Dunnett's procedure for comparing treatment means with control means.

Using Split Tomato Roots to Evaluate Translocation

The roots and stems of tomato plants about 15 cm tall were split into two approximately equal parts from the cotyledonary node down as far as possible on the tap root. Two 10 cm pots were taped together and the plant placed above the joined pots with half the root system in each pot. Potting soil was added and the plants staked for support. Two weeks after splitting, roots in one pot for each plant were treated. The other half of the root system remained in an untreated pot. Sets of five plants were thus treated with 200 μg oryzalin/pot, 50 μg oryzalin/pot, and 138 mg BAS 083 00W/pot. There was a set of five untreated control plants. Each control plant was split as the treated plants were split and knots from the 10 root halves were counted. One week after treatment all pots were inoculated with 2,000 nematode larvae per pot. Plants were harvested six weeks after inoculation.

Incubating Nematode Larvae in Treatment Solutions

Oryzalin did not dissolve well in water; therefore, water was added after oryzalin was dissolved in acetone. Nematode larvae were incubated for 24 hours in the following solutions: 400 μg oryzalin in 30 ml 4% acetone, 400 μg oryzalin in 30 ml 10% acetone, 2,000 μg oryzalin in 4% acetone (not completely soluble), 2,000 μg

oryzalin in 10% acetone, 4% acetone control, 10% acetone control, water control. After 24 hours, larvae were washed free of solution using a 400 mesh sieve, suspended in water, and observed under a microscope. Larvae incubated in 20 ppm oryzalin in 4% acetone were washed free of solution, resuspended in water, and used as a treatment in a split plot design experiment.

Nematode larvae were incubated for 24 hours in 50,000 ppm, 10,000 ppm, 5,000 ppm, and 2,500 ppm solutions of BAS 083 00W. After 24 hours, larvae were washed free of solution using a 400 mesh sieve, suspended in water, and observed under a microscope. Larvae incubated in the 10,000 ppm solution were inoculated on untreated plants as on "treatment" in the split plot design experiment. Untreated larvae were used as control. The third treatment in this experiment was the soil drench of 15 ml of 10,000 ppm BAS 083 00W per pot.

Transplanting Treated Plants to Untreated Soil Before Inoculation

Fifteen tomato seedlings were grown from seed for five weeks in vermiculite in 15 cm pots. One pot was treated with 700 mg BAS 083 00W in 50 ml water. Another pot was treated with 1,250 µg oryzalin in 50 ml 10% acetone. Two days after treatment five plants of each treatment (including an untreated control) were transplanted into new pots containing untreated potting soil.

Five days after transplanting, these plants were inoculated with 2,000 nematode larvae per pot. Plants were approximately 12 cm high at time of inoculation. Data were analyzed using Dunnett's procedure for comparing treatment means with the control mean.

Measuring Larval Growth

Five tomato plants approximately 15 cm high were treated with 200 µg/pot oryzalin and five plants were used as untreated controls. One week after treatment plants were inoculated with 2,000 larvae per plant. Twenty-four hours after inoculation plants were transplanted to uninoculated soil to fix the time of nematode penetration. At 7, 10, 13, 17, and 20 days following inoculation a pair of plants (one treated and one untreated) were harvested, roots fixed in FAA, and stained with acid fuchsin lactophenol. Larvae were dissected from the galls, arranged on slides, projected on tracing paper using a camera lucida attached to the microscope, and traced. The tracings were cut and area was measured to the nearest cm^2 using a leaf area analyzer. The area of the nematode silhouette in mm^2 was estimated by multiplying the tracing area by $1/4900$ because .1 mm on the slide micrometer was magnified to 7 cm on the tracing board.

A similar experiment was done with tomato plants treated with 138 mg/pot BAS 083 00W and harvested 8, 12, 16, and 20 days after inoculation.

Observing Larval Penetration in Cotton Roots

Blotter rolls of 'Deltapine M-8' cotton were prepared according to the method of McClure and Robertson (1973). Twenty-four hours after planting, the germinating seeds were treated with 10 ppm oryzalin. Twenty-four hours later, approximately 100 larvae per root tip were applied. Forty-eight hours after inoculation roots were harvested, stained with acid fuchsin in ethanol and acetic acid, cleared in chloral hydrate, and observed under the microscope in lactophenol. Selected root tips were photographed.

Special Experiments

The following experiments were done to gain a better understanding of the various methods of evaluating the roots for galling.

Variability Due to Knot Counting Alone

Five roots were selected to be counted six times each. The roots were representative of the kind of roots normally being evaluated and were counted in the standard way. The roots were counted randomly six different times by the same person without knowledge of origin. The mean

and standard deviation of the counts and of the square root of the counts were computed.

Bottle Cap Experiment

Fourteen untreated plants were inoculated with nematodes in the usual way. After six weeks the roots were harvested and knots counted. The knot counts were 553, 542, 916, 607, 243, 569, 472, 169, 492, 786, 490, 425, 496, and 474. Each knot count was written on a small bottle cap, placed in a box and mixed well. \underline{n} caps were drawn and the count recorded. The result was a crude model of an experiment run with \underline{n} control plants. The caps were returned to the box and the process repeated 20 times for $\underline{n} = 3$ and 10 times each for $\underline{n} = 5$ and $\underline{n} = 7$. Numbers drawn from the box were analyzed as if they were knot counts in regular experiments. The square root transformation applied and \bar{x} , σ , and σ/\bar{x} were computed.

RESULTS

Of the compounds tested, oryzalin and BAS 083 00W were most effective in reducing knot counts on nematode inoculated tomato roots. Results of experiments with these compounds are reported first. The following section deals with results that pertain to questions on methodology. The final section summarizes results of compounds other than oryzalin and BAS 083 00W.

Oryzalin and BAS 083 00W Studies

Oryzalin was tested in randomized complete block designed experiments with five replications per treatment and reduced knot counts over a wide range of concentrations. There was a reduction in root weight accompanying the reduction in knot counts, but the treatment of 250 μ g of oryzalin per pot reduced knot counts dramatically in comparison to the reduction in dry root weight (Table 2). In the experiment in which the time between treatment and inoculation was varied, knots were reduced greatly without root growth being affected (Fig. 1). Note also that oryzalin reduced knot counts even when applied one week after inoculation (Fig. 1).

The experiment in which tomato roots were split showed that oryzalin was not translocated. Treatment with

Table 2. Effect of oryzalin on knot counts, heights, and dry root weights of tomato.

Treatment in $\mu\text{g}/\text{pot}$ soil drench	Knot counts ^a		Heights (in cm)		Dry root weight (in gm)	
	Treated	Control	Treated	Control	Treated	Control
100	85 \pm 62*	180 \pm 54	n/a ^b	n/a ^b	.47	.63
250	23 \pm 29**	962 \pm 88	58	66	.47	1.20
500	51 \pm 13**	200 \pm 33	43	63	.33	.55
1000	4 \pm 7**	200 \pm 33	20	63	.21	.55

^a* and ** indicate significant differences from control for knot counts at the 5% and 1% level by Dunnett's Test.

^bData not available.

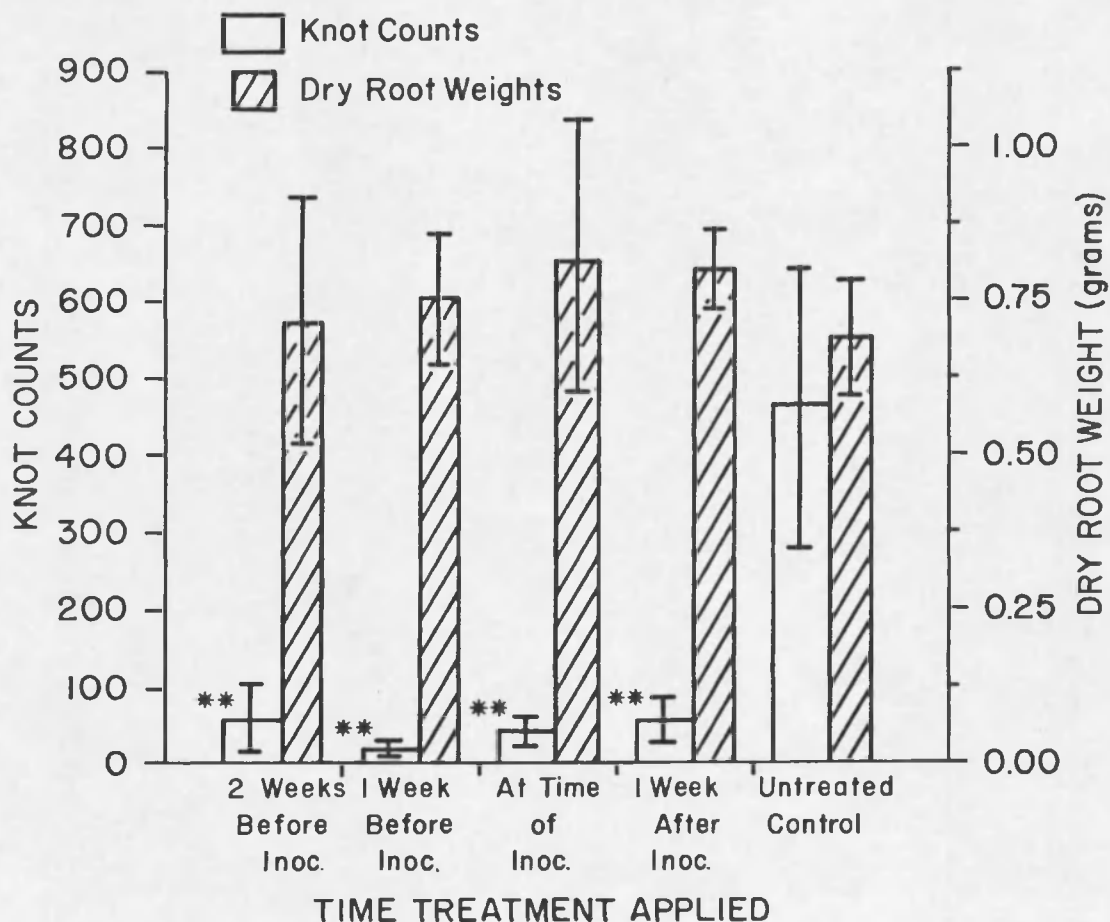


Fig. 1. Varying the time between oryzalin treatment and inoculation -- Knot counts were reduced whereas dry root weights were not for each time of treatment. The lines indicate standard deviation and ** indicates significant difference from control at the 1% level by Dunnett's Test.

both 200 $\mu\text{g}/\text{pot}$ and 50 $\mu\text{g}/\text{pot}$ oryzalin reduced knot counts on the treated side of the plant but not on the untreated side (Fig. 2). The main purpose of the split root experiment was to test if the effect of the chemical was translocated, but in addition, comparing the treated and untreated sides of the same plant showed that oryzalin reduced knot counts on roots with identical genes and the same above ground environment.

The effect of oryzalin was on the plant rather than on the larvae directly. Incubating the larvae for 24 hours in 20 ppm oryzalin in 4% acetone did not reduce their ability to infect roots (Fig. 3). Microscopic observation of larvae soaked in 4% and 10% acetone showed that acetone had a paralyzing effect on the larvae. Washed free of 4% acetone and resuspended in water, most larvae regained mobility in a few hours. Washed free of 10% acetone and resuspended in water, not all larvae recovered. There were no differences between larvae incubated in oryzalin solutions and the corresponding acetone controls. Plants grown for two days in an oryzalin treated pot and then transplanted to untreated soil five days prior to inoculation still reduced knots as compared to controls (Fig. 4).

Growth of larvae in oryzalin treated plants was not affected by treatment based on larvae that could be found (Fig. 5). No incipient galls or larvae could be found in treated roots harvested 7 and 10 days after inoculation and

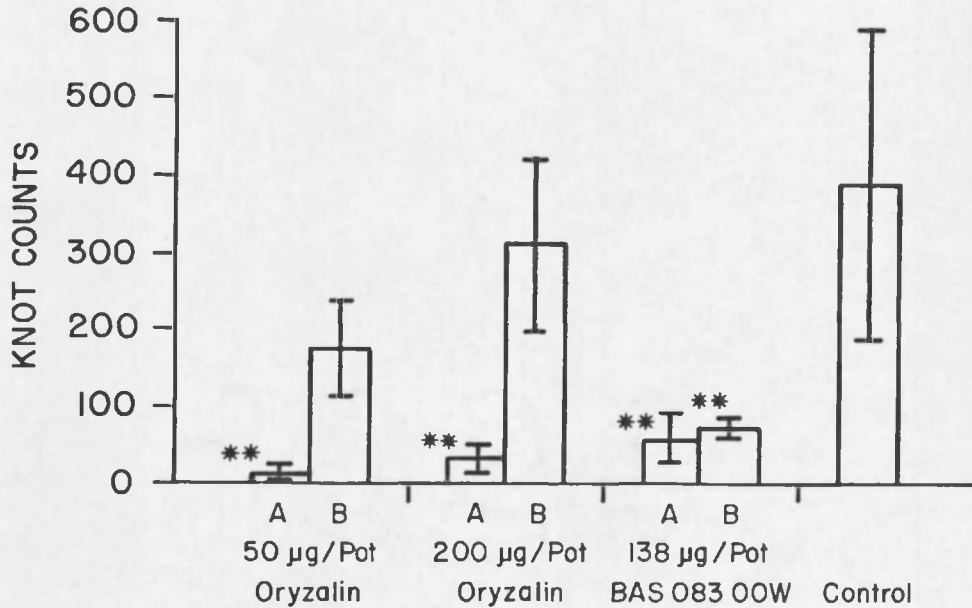


Fig. 2. Split tomato roots to evaluate translocation -- BAS 083 OOW was translocated, but oryzalin was not. Knot counts from the treated side of split roots are labeled A; those from the untreated side are labeled B. The lines indicate standard deviation and ** indicates significant differences at the 1% level by Dunnett's Test.

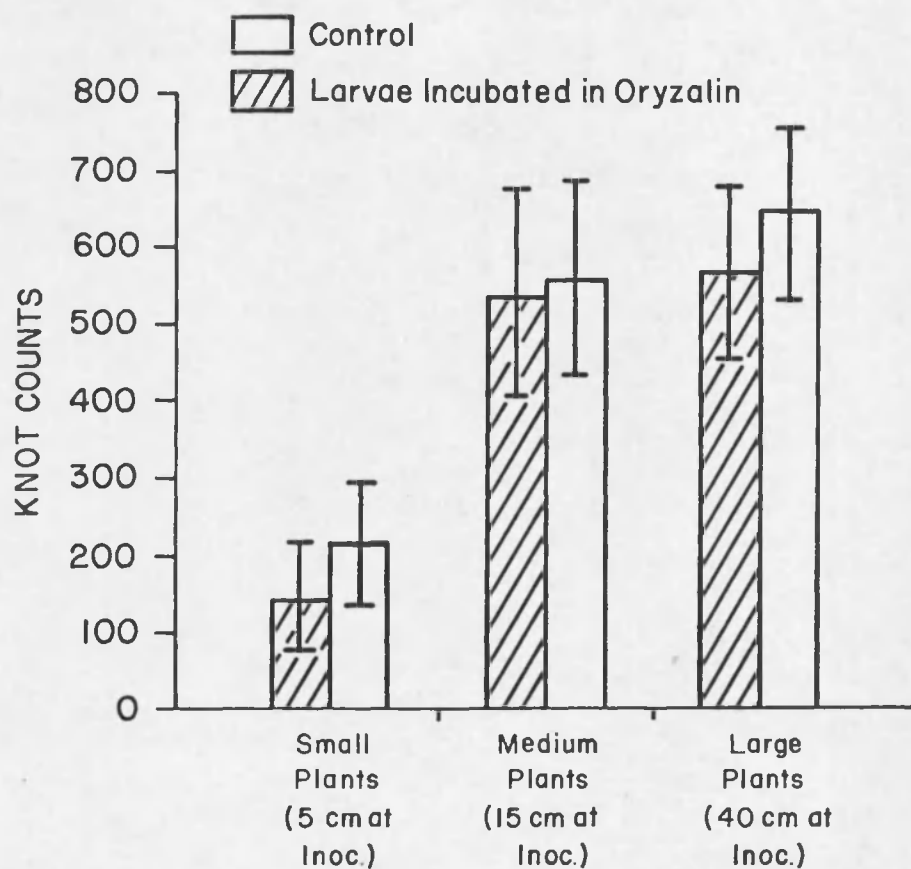


Fig. 3. Incubating larvae in 20 ppm oryzalin for 24 hours prior to inoculation -- Oryzalin was not toxic to the nematodes on direct contact. The lines indicate standard deviation. Differences are not significant at the 5% level by Dunnett's Test.

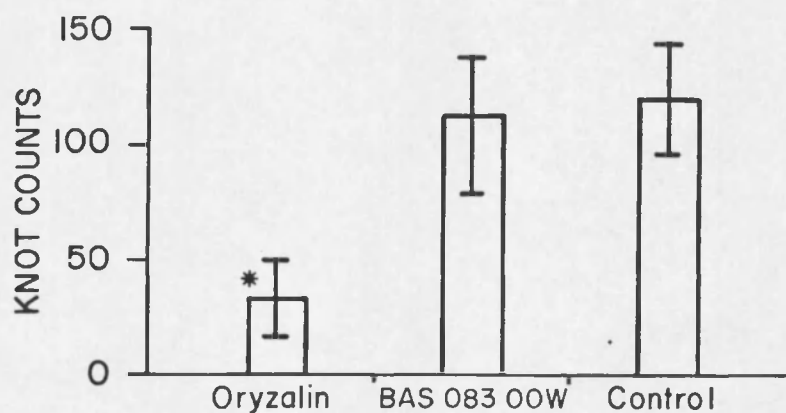


Fig. 4. Transplanting treated plants to untreated soil before inoculation -- Oryzalin, but not BAS 083 OOW, reduced knot counts in this experiment. The lines indicate standard deviation and * indicates significant difference from control at the 5% level by Dunnett's Test.

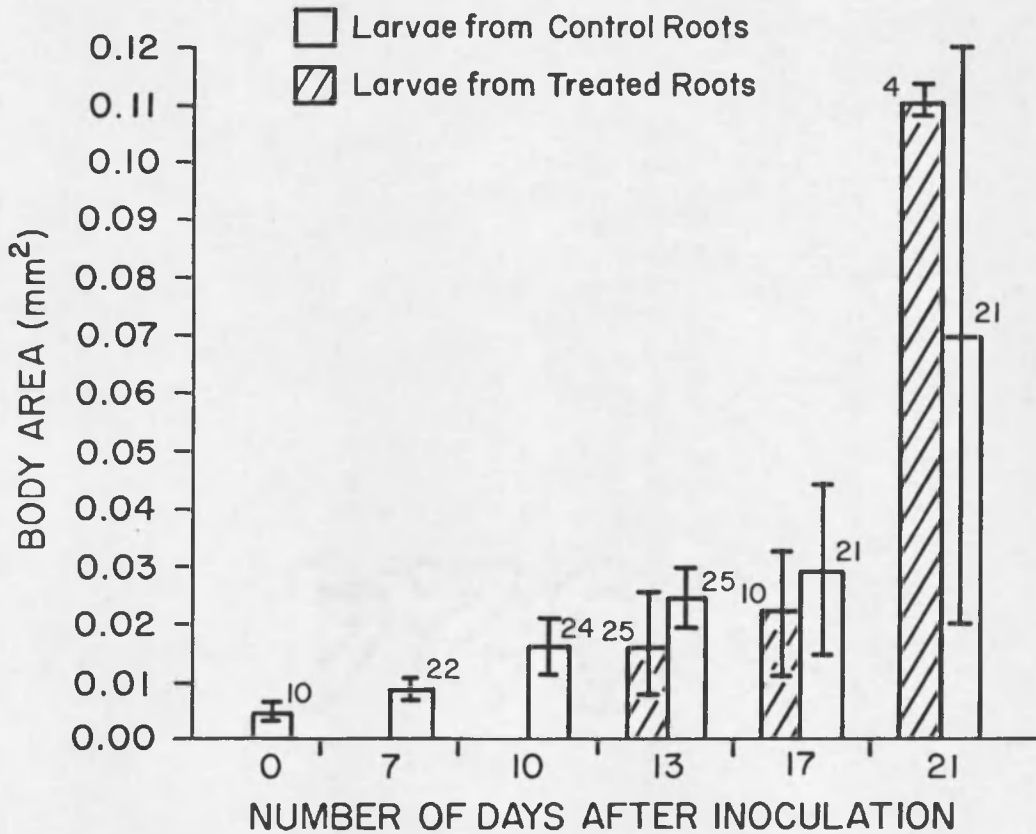


Fig. 5. Effect of oryzalin on growth of nematode larvae -- Body area of larvae found in oryzalin treated roots was not different from body area of larvae in control roots, but the sample from treated roots was small. The numbers above the bars indicate how many larvae were measured and the lines indicate standard deviation. No larvae were found on treated roots at 7 and 10 days.

only four galls could be found in the 14 day root. The four galls in this root had many larvae in them and 25 were dissected out. The treated plant harvested 21 days after treatment also had only four galls. In this case, there was one adult female in each gall. By contrast to the treated roots, galls and nematodes were abundant in the control plants and it was easy to find 20 to 25 nematodes to be traced. One effect oryzalin had on penetration was to stop root tip growth and confine many larvae to a small area (Fig. 6).

Another compound that effectively reduced nematode activity in tomato plants was BAS 083 00W which lowered knot counts, egg counts, and gall index (Table 3). The reduction in all three measures for the middle and large size plants is convincing. Note the reduction in both height and root weight in treated plants. In the larger plants, the reduction in height and root weight was not severe and yet there was a reduction in knots from approximately 800 in controls to 100 in treatment.

The rate of application of BAS 083 00W was 138 mg/pot (410 kg/ha), which was high. Following treatment, leaves of plants turned dark green and lateral root growth was inhibited. In the experiment in which the time between treatment and inoculation was varied (Fig. 7), there was not the reduction in knot counts seen in Table 3. Plants treated two weeks and one week prior to inoculation had

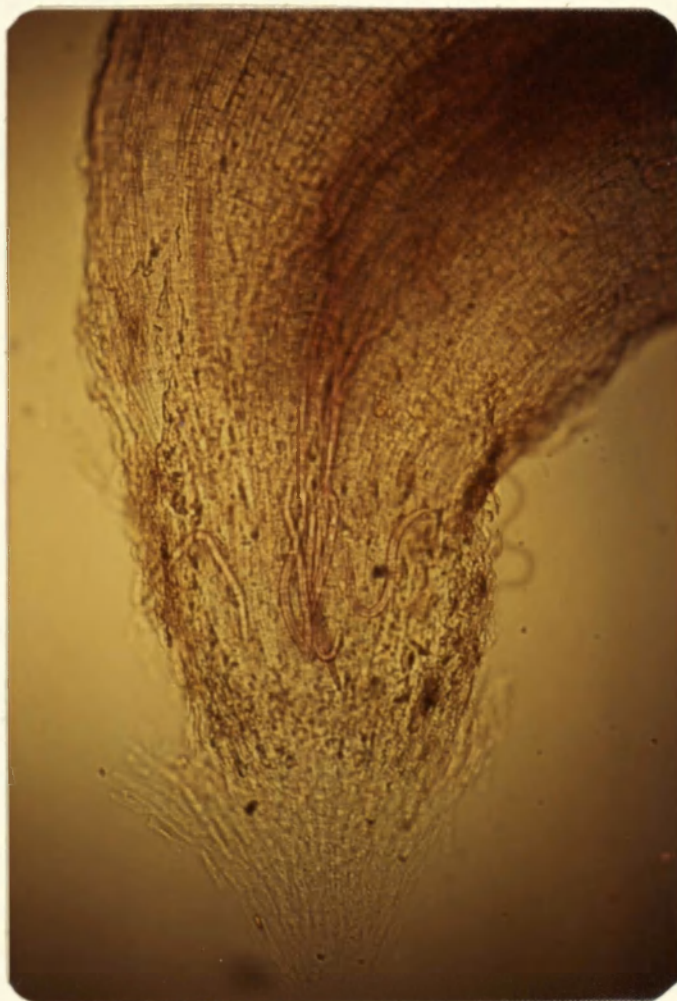


Fig. 6. Effect of oryzalin on penetration of nematode larvae into a cotton root tip -- The nematodes are confined to a small area at the tip of the root. Tissue just behind the tip is hypertrophied and disorganized by the treatment with oryzalin.

Table 3. Effect of BAS 083 OOW on knot counts, egg counts, gall index, dry root weight, and heights of tomato plants.

Heights of plants at time of treatment (cm)	Knot counts		Knot counts		Egg counts		Dry root weight (grm)		Heights (cm)	
	Knot counts		Treat-ment	Control	Treat-ment	Control	Treat-ment	Control	Treat-ment	Control
	Treatment	Control								
34	102+70	799+149	33	241	2	7	.75	.95	46	53
12	174+75	926+79	97	358	3	7	.46	.82	38	65
5	15+13	84+26	6	27	1	3	.06	.09	10	31

Treatment was by soil drench of 138 mg/pot. Each entry is the mean of 5 observations.

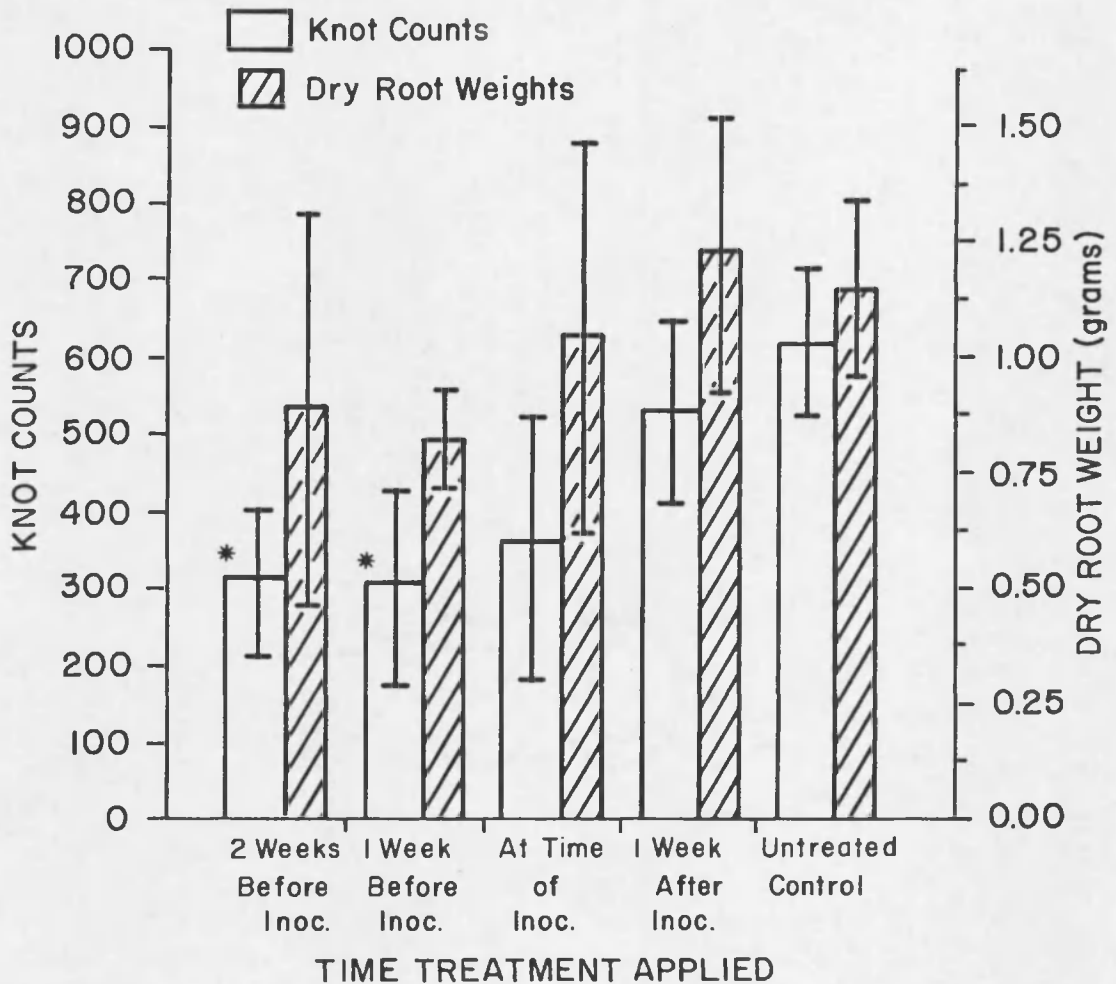



Fig. 7. Varying the time between BAS 083 OOW treatment and inoculation -- Knot counts were reduced when treatment was applied one and two weeks before inoculation. The lines indicate standard deviation and * indicates significant difference from control at the 5% level by Dunnett's Test.

fewer knots than controls. Plants treated one week after inoculation were not different from control plants. In the split root experiment, both the treated and the untreated sides of treated plants showed significantly fewer knots than control plants (Fig. 2).

Incubating larvae in 10,000 ppm BAS 083 00W for 24 hours prior to inoculation reduced their ability to infect the tomato roots by about half for each size plant (Fig. 8). There were over 400 knots on large plants inoculated with treated larvae. Of the larvae treated, a substantial number were able to infect plants. Notice also that plants treated with 15 ml of 10,000 ppm BAS 083 00W one week prior to inoculation showed significantly fewer knots than plants inoculated with larvae incubated in this same concentration. Microscopic examination showed that larvae incubated in 5,000 ppm and 2,500 ppm BAS 083 00W were affected less than larvae incubated in 10,000 ppm. Treated plants transplanted to untreated soil before inoculation were infected at the same level as control plants (Fig. 4). Larvae that penetrated roots of treated plants developed at the same rate as larvae that penetrated roots of untreated plants (Fig. 9).



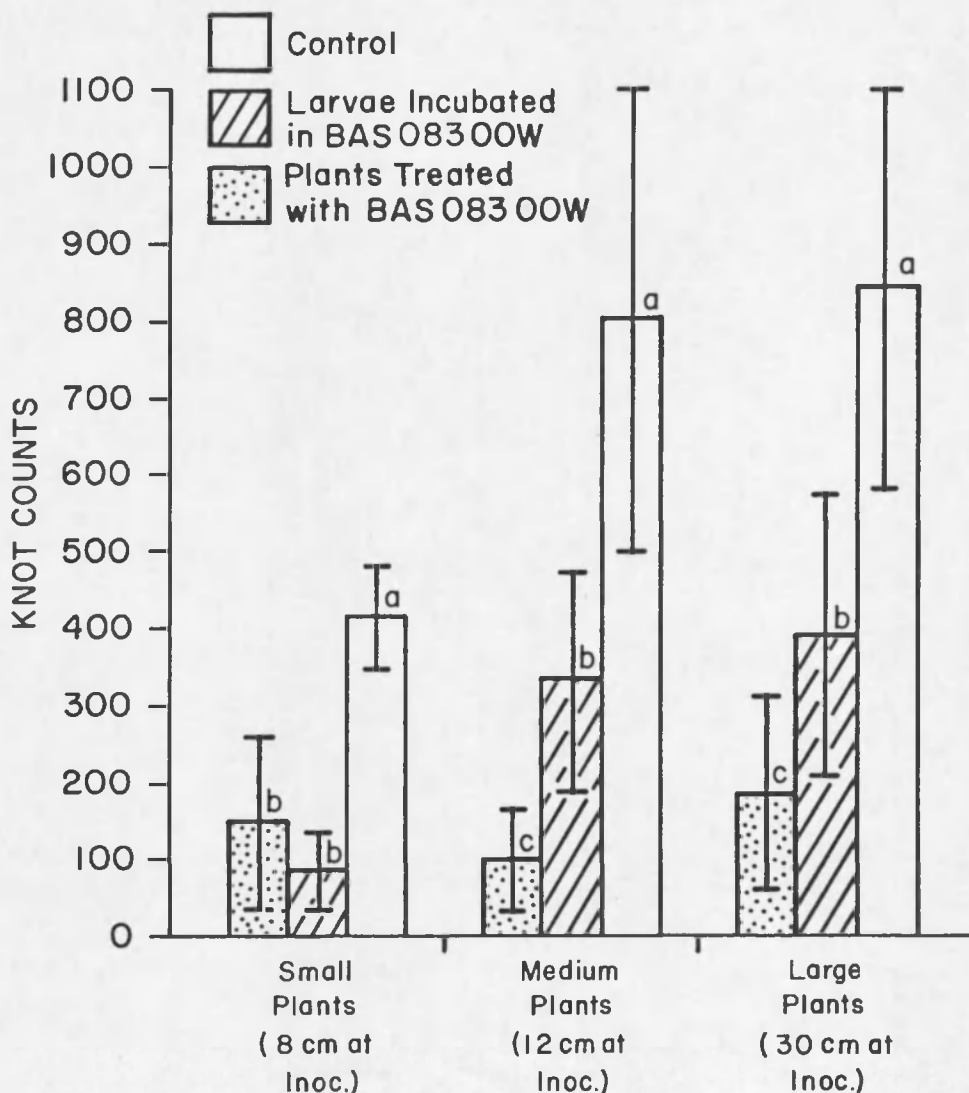


Fig. 8. Incubating larvae in 10,000 ppm BAS 083 OOW for 24 hours prior to inoculation -- BAS 083 OOW was toxic to the nematodes on direct contact. Knot counts on medium and large plants were reduced more when the plants were treated than when the larvae were treated directly. The lines indicate standard deviation. Within each plant size, bars labeled with the same letter do not differ significantly at the 5% level by Duncan's Multiple Range Test.

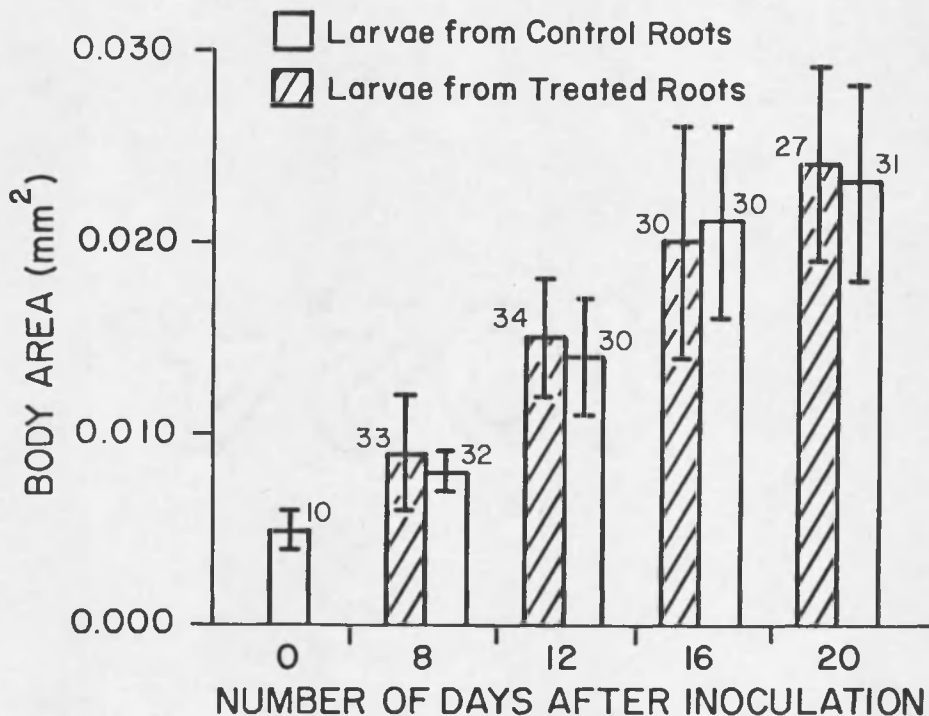


Fig. 9. Effect of BAS 083 OOW on growth of nematode larvae -- Body area of larvae found in BAS 083 OOW treated roots was not different from body area of larvae in control roots. The numbers above the bars indicate how many larvae were measured, and the lines indicate standard deviation.

Results That Pertain to Methodology

Knot Counts

Variability in knot counts made interpretation of data difficult when knot counts were low. Control plants with high knot counts demonstrated treatment effects more clearly than control plants with low knot counts (Table 4). In seven split plot designed experiments, the coefficient of variability (σ/\bar{x}) was about 30% for root systems with less than 250 knots and only about 20% for root systems with over 450 knots. An attempt to estimate the error due to counting alone indicated that the counting error for the high knot counts was less than for the small knot counts (Table 5).

The coefficient of variability (CV) ranged from 7% to 30% for individual experiments where the square root transformation was applied. The bottle cap experiment was designed to model the variability as measured by σ/\bar{x} when the number of replications was 3, 5, and 7 (Table 6). Notice how the lower end of the range of σ rises as the number of replications increases. Also notice the lower end of the range of σ/\bar{x} . The data transformed by square root is not displayed because the model is for intuitive rather than rigorous demonstration. σ/\bar{x} was reduced by very nearly one-half in each case when the data were transformed by square root. Compare draw 2 and draw 8 to see

Table 4. Control plants with high and low knot counts.

Experiment	Control	Treatment	Kind of treatment
1	68+ <u>20</u>	20+ <u>16</u>	500 μ g/pot Oryzalin
2	727+ <u>170</u>	20+ <u>19</u>	200 μ g/pot Oryzalin

Table 5. Variability due to counting alone.

Sample root number	$\bar{x} + \sigma$ for six counts of the same sample root	σ / \bar{x}
1	653+ <u>104</u>	16%
2	483+ <u>79</u>	16%
3	363+ <u>83</u>	23%
4	330+ <u>64</u>	19%
5	222+ <u>66</u>	30%

Table 6. Results from bottle cap experiment.

Initial population:

553	542	916	607	243
569	472	169	786	490
425	496	474	492	

Number of replications	Range of \bar{x}	Range of σ	Range of σ/\bar{x}
3	369 ↔ 655	39 ↔ 283	7 ↔ 77%
5	393 ↔ 618	55 ↔ 223	10 ↔ 43%
7	456 ↔ 627	105 ↔ 247	29 ↔ 50%

Extreme examples from experiments modeled with five replications:

	Bottle caps drawn					\bar{x}	σ	σ/\bar{x}
Draw 2	786	553	569	496	169	515	223	43%
Draw 8	490	553	474	569	607	538	55	10%

the difference in variability due simply to chance when the number of replications is five (Table 6). In summary, five replications gives a reasonable estimation of the mean but can give misleadingly low estimations of the variability.

Gall Index

The gall index can be used as a quicker method to evaluate roots. Oryzalin treated roots were easily and consistently distinguished from control roots (Table 7). Ratings of 90 roots by two different people independently gave a high correlation (Fig. 10). The correlation is sufficiently high to indicate that different people would come to the same conclusion in distinguishing the kinds of differences observed in Table 7.

Egg Counts

The scattergram of egg counts with knot counts for 81 sets of roots shows a good correlation (Fig. 11). The slope of the regression line is .26. Since the total number of eggs recovered per root system can be estimated at 400 times the egg count, the slope indicates that the average number of eggs recovered per knot counted is about 100.

Plant Height at Time of Inoculation

Plant height in the tomato plant can be taken as a rough reflection of root mass. A scattergram of plant

Table 7. Gall index of treated and control roots when treatment is effective.

Treatment ^a	Gall index				
Gall index of twenty treated roots:					
a	1	1	2	1	4
b	0	1	1	0	1
c	1	1	2	1	1
d	0	0	1	1	1
Gall index of the five control roots each rated five times: ^b					
1st Rating	7	4	4	5	6
2nd Rating	6	6	4	4	7
3rd Rating	6	5	4	4	7
rth Rating	7	6	4	4	6
5th Rating	7	4	4	4	6

^aTreatment with oryzalin as described for Fig. 1. Knot counts and dry root weights for these roots are shown in Fig. 1.

^bAll ratings were done with the person rating not knowing which root was being rated. Control roots were interspersed with treated roots.

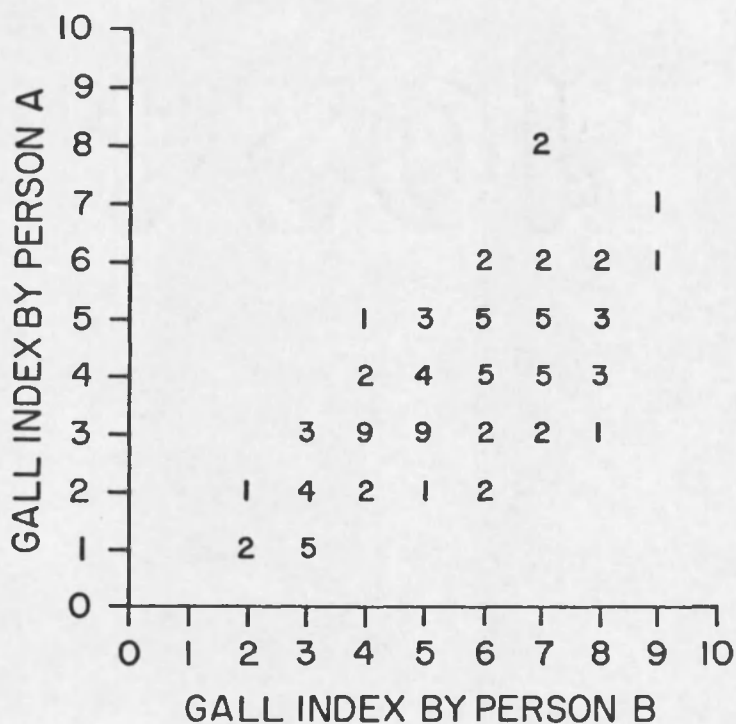


Fig. 10. Scattergram of gall indices for 90 roots rated by two people -- The correlation (R) is .74; R squared is .54; and the slope of the regression line is .64. The correlation is high enough to expect that large treatment differences can be detected using the gall index independent of the person doing the rating. The numbers on the graph indicate how many roots were rated by the coordinate of the point where the number is written.

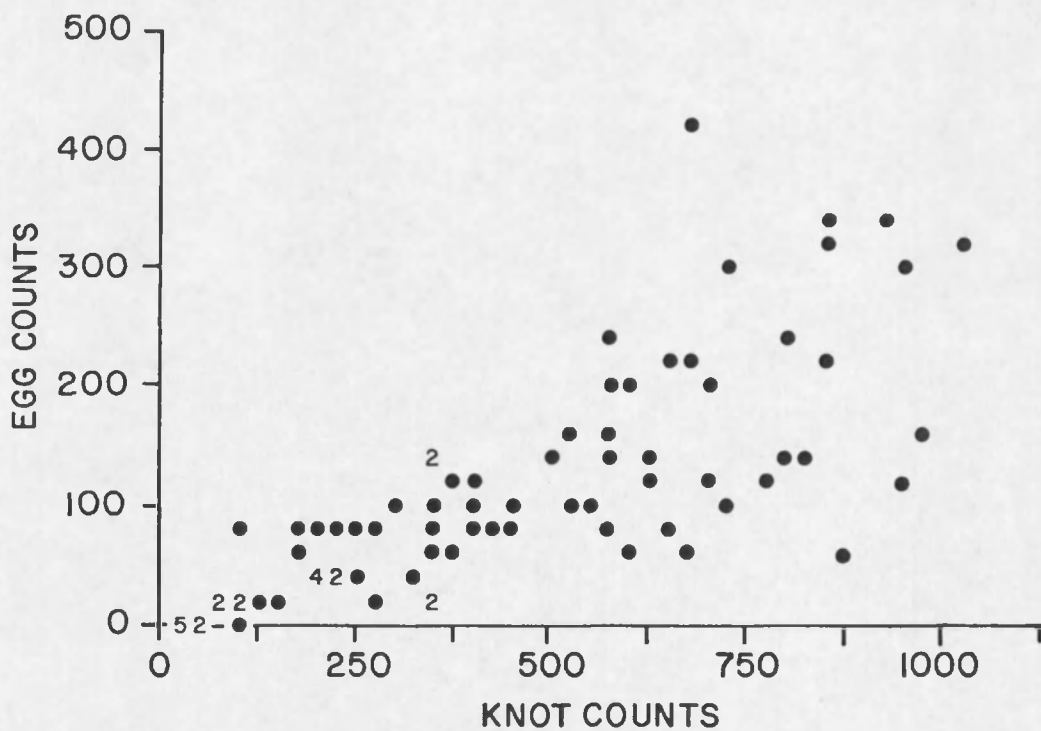


Fig. 11. Scattergram of egg counts with knot counts -- The correlation (R) is .77; R squared is .59; and the slope of the regression line is .26. The correlation is high enough to expect that egg counts can be used as effectively as knot counts for evaluation of roots.

height at inoculation with knot counts shows that below 12 cm, plant height can be a limiting factor on the number of knots in the roots (Fig. 12). In addition, there were significantly fewer knots on small control plants than large control plants in every split plot designed experiment.

Arrangement of Pots on the Greenhouse Bench

The analysis of variance in ten experiments using split plot design indicated that arrangement of pots on the greenhouse bench did not significantly affect knot counts, gall index, dry root weight, or plant height.

Summary of Additional Results

A number of chemicals gave no reduction in knot counts over a wide range of concentrations (Table 8). Others gave a reduction in knot counts at rates which caused severe root damage or pruning (Table 9). Abscisic acid gave a reduction in knot counts in some completely randomized block designed experiments at 500 µg/pot. The inability to repeat these results in many trials indicated that the differences were probably due to chance. Chemicals used in foliar dip treatments did not give significant reductions in knot counts with the exception of BAS 083 00W (Table 10).

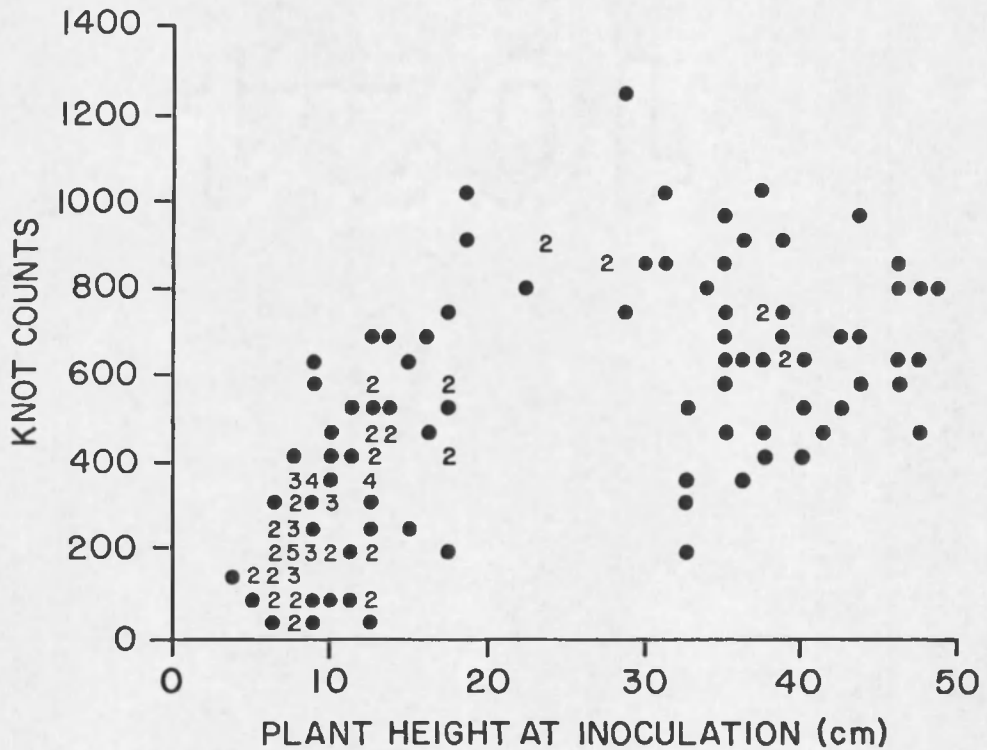


Fig. 12. Scattergram of knot counts with heights at inoculation -- The correlation (R) is .68; R squared is .46; and the slope of the regression line is 13.6. Below 12 cm plant height at inoculation was associated with a limiting factor in the number of knots formed.

Table 8. Chemicals which gave no reduction in knot counts when applied as a soil drench.

Name of chemical	Rate (in amount ai/pot)
EPTC	.5 µg, 5.5 µg, 55 µg, 550 µg, 1800 µg, 3000 µg, 3600 µg, 5500 µg
Glyphosine	40 µg, 400 µg, 500 µg, 600 µg, 700 µg, 4000 µg, 10000 µg
IAA	500 µg, 1000 µg
GA	200 µg, 400 µg, 600 µg, 800 µg
Kinetin	200 µg, 400 µg, 800 µg, 1000 µg, 1200 µg
Virazole	50 µg, 500 µg, 1000 µg, 5000 µg, 10000 µg
Cytex	500 µg
Abscisic Acid	200 µg, 300 µg, 500 µg, 600 µg, 900 µg, 1000 µg, 2000 µg, 10000 µg

Table 9. Chemicals which reduced knot counts at rates which caused root damage.

Name of chemical	Rate (ai/pot)	Knot counts		Dry root weight (grm)	
		Treat-ment	Control	Treat-ment	Control
Glyphosine	20 mg	62	199	.22	.76
MH	5 mg	16	150	.16	.33
NAA	1 mg	88	284	.14	.54
ABA	15 mg	43	132	.22	.43
BAPN	500 mg	16	193	.05	.30
Phosphon	10 mg	41	103	.09	.31
Ethrel	140 mg	14	228	.06	.30
Ancymidol	2 mg	11	382	.03	.38

All treatments were by soil drench. Each entry is the mean of five observations.

Table 10. Knot counts for treatments by foliar dip.

Chemical	Concentration of the a.i. in the dip	Knot counts	
		Treatment	Control
BAS 083 OOW	4600 ppm	608*	799
		581*	926
		69	84
Oryzalin	5 ppm	646	644
		654	556
		122	212
Phosphon	1000 ppm	517	492
		373	430
		91	103
Ethrel	1000 ppm	519	614
		548	568
		191	228
Cytex	.5 ppm	694	675
		641	573
		189	197

Each entry is the mean of five observations. * indicates the difference between treatment and control is significant at the 5% level using Duncan's Multiple Range Test.

DISCUSSION

Feasibility of Increasing Plant Resistance

The feasibility of increasing plant resistance to root knot nematodes using plant growth regulators was demonstrated by the examples of oryzalin and BAS 083 00W. These chemicals reduced knot counts on treated tomato plants (Tables 2 and 3). Results of the split root experiment (Fig. 2) and experiments in which the time between treatment and inoculation was varied (Figs. 1 and 7) confirmed that roots treated with these compounds had fewer knots. In these tests, both oryzalin and BAS 083 00W exhibited greater in vivo activity than in vitro toxicity would predict (Figs. 3 and 8). The results from these two compounds followed a pattern reported for other plant growth regulating compounds on nematode infected plants: maleic hydrazide (MH) (Davide and Triantaphyllou 1968); thiourea, actidone, TIBA (Kochba and Samish 1972); morphactin (Orion and Minz 1971); and DCPA (Romney et al. 1974).

In the case of oryzalin, it was apparent that there was an effect on penetration or nematode activity immediately after penetration (Fig. 6). Romney et al. (1974) suggested that DCPA worked by inhibiting penetration;

however, 200 µg/pot oryzalin reduced knot counts when applied a week following inoculation (Fig. 1). Clearly, more than penetration was affected because by one week after inoculation many larvae had already penetrated. In the larval growth experiments, many larvae penetrated control roots within 24 hours of inoculation. Davide and Triantaphyllou (1968) reported a similar occurrence with MH. Treatment by MH prior to inoculation prevented penetration. Treatment after inoculation reduced giant cell development, increased the number of males in the population, and reduced galling. It is possible, therefore, that oryzalin is acting in two stages of the infection process: penetration and post penetration development.

Oryzalin affects mitosis by preventing the formation of spindle microtubules. The result is large polyploid nuclei and sometimes multinucleate cells. These cells appear very different from the highly organized multinucleate giant cells found in response to the root knot nematode (Bartels, 1977). Orion and Minz (1971) emphasized that nematode development is dependent upon the giant cells. They provided evidence that morphactin, a plant growth regulator affecting mitosis, interfered with giant cell formation and thereby nematode development. The giant cells in morphactin treated plants were smaller and had fewer nuclei than giant cells in control plants. The examination of cotton root tips treated with oryzalin

showed that cellular organization was greatly disrupted (Fig. 6). Reduction in knots when plants were treated after inoculation (Fig. 1) could also be explained by the inhibition of mitosis: an interference with the formation of normal giant cells and subsequent nematode development.

Although oryzalin was effective in reducing nematode activity, more nematicidal compounds might be found. The range at which oryzalin was effective was just below herbicidal levels. Plants 6 cm high (5 weeks old) or less were severely damaged or killed by oryzalin. In a related experiment, four week old tomato seedlings treated with trifluralin (also a dinitroaniline compound) and inoculated with Meloidogyne hapla had much less root growth than inoculated seedlings grown in untreated soil, but galling was not evaluated (Anderson and Griffin, 1977). Chances of damage to small plants limit oryzalin's desirability as a control compound. Plants 12 cm or taller at the time of treatment were not severely damaged by oryzalin. The fact that reduction in knot counts occurred without root damage in some cases (Fig. 1) suggests the possibility that the chemotherapeutic aspects of oryzalin might be separated from its herbicidal activities.

A compound which is systemic is more desirable for control than one which is not. Oryzalin was not translocated in the split root experiment (Fig. 2). The split root experiment results are consistent with the fact that

dinitroaniline compounds such as oryzalin are not translocated (Strang and Rogers 1971). A compound which is as effective as oryzalin, but which is translocated, would be an improvement.

BAS 083 00W results contrasted with the oryzalin results. Both compounds reduced galling (Tables 2 and 3), but BAS 083 00W did not reduce galling when applied a week after inoculation (Fig. 7), whereas oryzalin did (Fig. 1). BAS 083 00W reduced galling on both the untreated and treated sides of split root plants, whereas oryzalin did not (Fig. 2). BAS 083 00W reduced infectivity of larvae (Fig. 8), whereas oryzalin did not (Fig. 3). BAS 083 00W did not reduce galling when treated plants were transplanted to untreated soil; oryzalin did (Fig. 4).

Large and medium sized plants treated with BAS 083 00W had significantly fewer knots than untreated plants inoculated with treated larvae (Fig. 8). Although BAS 083 00W is somewhat toxic to nematodes at 10,000 ppm, the toxicity is not great enough to account for the reduction in knots when plants are treated one week prior to inoculation. In the split root experiment, for example, it would be surprising if BAS 083 00W was translocated to the untreated side in concentrations large enough to cause the reduction in knots observed (Fig. 2).

If BAS 083 00W is not acting as a contact nematocide, then it is acting through the plant in some way. A

possible explanation of BAS 083 00W action is suggested by the observation that BAS 083 00W treated plants had fewer lateral roots than control plants. Fewer lateral roots would mean fewer sites for penetration by the nematode. The scattergram showing the relationship of plant height at the time of treatment with knot counts indicated that sites of penetration could be a limiting factor in the number of knots (Fig. 12). The reduction in lateral roots might also mean that the chemical was active in the pericycle of the root where lateral roots are initiated. Since pericycle and adjacent tissues are the sites of giant cell formation, alterations of developing giant cells by BAS 083 00W could be expected to influence nematode growth and reproduction.

The results of oryzalin and BAS 083 00W should be viewed in the context of the many treatments which gave no reduction in knot counts at any concentration tested (Tables 10 and 12), as well as those treatments which reduced knot counts but also caused severe root damage (Table 11). The review of the literature demonstrates that chemical modification of plants to reduce pest infestation is a widely reported laboratory phenomenon. Results of oryzalin and BAS 083 00W are consistent with this pattern and suggest that it might be feasible to use plant growth regulators to increase plant resistance.

Comments on Methodology and
Screening

One of the goals of the research was to suggest techniques by which a large number of compounds might be screened effectively for increasing plant resistance to root knot nematode. Such a screening program must be operated under constraints of expense for labor, equipment, greenhouse space, and other facilities.

Conventional techniques for evaluating nematode infection of tomato plants include counting knots and using a gall index. Knot counting was the primary technique used in this work, and although variability was high, it was possible to detect successful treatments without difficulty when control plants had 300 to 400 knots. Counting a root system takes approximately ten minutes and therefore it would require approximately one working day to evaluate 50 roots using this method. A quicker method--also capable of detecting differences--is the gall index (Table 7), whereby 50 roots can be evaluated in approximately one hour.

Veech (1977) has reported an insecticide that reduced egg production in root knot nematode infested seedlings without reducing galling. Kochba and Samish (1972) found that actidone in low concentrations inhibited development of females, but did not inhibit galling. Such treatments would be detectable by counting eggs and not by counting knots.

Evaluation of egg production takes place at the end of the nematode life cycle and therefore would detect deleterious effects at any stage of the nematode's life cycle. There is sufficient correlation between knot counts and egg counts for one to expect that any reduction in knot counts would also be reflected in the number of eggs counted (Fig. 11). Inoculation of tomato plants 12 cm to 16 cm high with 2000 larvae per plant usually results in about 400 knots per root (Fig. 12). The estimation of 100 eggs per knot counted would imply that were all eggs recovered and concentrated as many as 40,000 eggs could be expected from one root system. In a large scale evaluation program based on egg counts, it would be desirable and possible to obtain clean egg samples using the method of McClure, Kruk, and Misaghi (1973).

The effect of a successful treatment on egg production was enough to expect that two plants per chemical treatment would be sufficient for a primary screen (Table 3). Position of pots on the greenhouse bench was not a critical variable; however, plants must be spaced to allow for growth. Two hundred plants in 10 cm pots can be accommodated on a 2 m by 3 m bench. To evaluate 10,000 treatments per year with 2 plants per treatment would require twenty-two 2 m by 3 m greenhouse benches to allow for an eleven week cycle following transplanting.

In a large screening program, there is heavy pressure to use plants which are as small as possible in order to save space and to avoid greenhouse problems associated with large plants. Evaluation of egg production as suggested requires large plants. In addition to the desirability of evaluating eggs, there is a second reason for using larger plants in the primary screen. The greatest potential use for plant growth regulators to control nematodes may well be with perennials and in other situations where soil fumigation is not possible. By screening plants which are too small by way of convenience, the most valuable compounds might be missed. Oryzalin is an example of such a compound.

In secondary testing it would be useful to vary the time between treatment and inoculation. Treating plants one week before, at the time of, and one week after inoculation can distinguish between pre-penetration and post-penetration effects on the nematode (Figs. 1 and 7). More replications should be included in the secondary testing and roots could be evaluated by gall index or knot count as well as egg count. This would determine if eggs were reduced with or without a reduction in galling. In both primary and secondary testing, oryzalin treatment could be included as a standard for comparison in addition to the untreated controls.

LITERATURE CITED

- Allen, T. C. 1947. Suppression of insect damage by means of plant hormones. *J. Econ. Entomol.* 40:814-817.
- Altman, J., and M. Ross. 1967. Plant pathogens as a possible factor in unexpected preplant herbicide damage in sugarbeets. *Plant Dis. Rep.* 51:86-88.
- Anderson, J. L., and G. D. Griffin. 1972. Interaction of DCPA and Trifluralin with seedling infection by root-knot nematode. *Abstr. Weed Sci. Soc. Amer.* 11-12.
- Anderson, J. L., and G. D. Griffin. 1977. Unpublished report, in possession of Dr. P. G. Bartels, Department of Plant Sciences, University of Arizona.
- Banks, D. J. 1970. Response of nematode infected peanut plants to maleic hydrazide. *Agron. J.* 62:812-815.
- Bartels, P. G. 1977. Associate Professor of Plant Sciences, University of Arizona, personal communication.
- Beckman, C. H. 1958. Growth inhibition as a mechanism in Dutch Elm Disease therapy. *Phytopathology*, 48: 172-176.
- Biehn, W. L., and A. E. Dimond. 1973. Chemically induced root injury correlated with a reduction of Fusarium wilt of tomato. *Phytopathology*, 63:655-656.
- Buchenauer, H., and D. C. Erwin. 1976. Effect of the plant growth retardant Pydanon on Verticillium wilt of cotton and tomato. *Phytopathology* 66: 1140-1143.
- Bunt, J. A. 1975. Effect and Mode of Action of Some Systemic Nematicides. H. Veenman en Zonen B. V., Wageningen, The Netherlands. 127p.
- Chappell, W. E., and L. T. Miller. 1957. The effect of certain herbicides on plant pathogens. *Plant Dis. Rep.* 40:52-56.

- Corden, M. E., and A. E. Dimond. 1959. The effect of growth regulating substances on disease resistance and plant growth. *Phytopathology* 49:68-72.
- Crittenden, H. W. 1954. Factors associated with root-knot nematode resistance in soybeans. Abstr. *Phytopathology* 44:388.
- Crossan, D. F., and D. J. Fieldhouse. 1964. A comparison of dwarfing and other compounds, with and without fixed copper fungicide, for control of bacterial spot of pepper. *Plant Dis. Rep.* 48:549-550.
- Davide, R. G., and A. C. Triantaphyllou. 1968. Influence of the environment on development and sex differentiation of root-knot nematode. III. Effect of foliar application of maleic hydrazide. *Nematologica* 14:37-46.
- Davis, David, and A. E. Dimond. 1953. Inducing disease resistance with plant growth regulators. *Phytopathology* 43:137-140.
- Dropkin, V. H., and W. R. Boone. 1966. Analysis of host parasite relationships of root-knot nematodes by single larva inoculations of excised tomato roots. *Nematologica* 12:225-236.
- Dropkin, V. H., J. P. Helgeson, and C. D. Upper. 1969. The hypersensitivity reaction of tomato resistance to Meloidogyne incognita; reversal by cytokinins. *J. Nematol.* 1:55-61.
- Erwin, D. C., S. D. Tsai, and R. A. Khan. 1976. Reduction of the severity of *Verticillium* wilt of cotton by the growth retardant tributyl ((5-chloro-2-thienyl)methyl) phosphonium chloride. *Phytopathology* 66:106-110.
- Feldman, A. W., and R. W. Hanks. 1963. Attempts at eradication of the burrowing nematode, Radopholus similis, from grapefruit seedlings by foliage applications of maleic hydrazide. *Plant Dis. Rep.* 47:27-29.
- Hartman, R. T., and W. P. Price. 1950. Synergistic effect of plant growth substances and southern bean mosaic virus. *Am. J. Bot.* 37:820-828.

- Hsia, Y. T., and J. J. Christensen. 1951. Effect of 2,4-D on seedling blight of wheat caused by Helminthosporium sativum. Phytopathology 41:1011-1020.
- Johnson, A. W., C. C. Dowler, and E. W. Hauser. 1975. Crop rotation and herbicide effects on population densities of plant-parasitic nematodes. J. Nematol. 7:158-168.
- Katan, J., and Y. Eshel. 1973. Interactions between herbicides and plant pathogens. Residue Rev. 45:145-177.
- Kiraly, Z., and J. Szirmai. 1964. The influence of kinetin on Tobacco Mosaic Virus production in Nicotiana glutinosa leaf discs. Virology 23:286-288.
- Kochba, J., and R. M. Samish. 1971. Effect of kinetin and 1-naphthylacetic acid on root knot nematodes in resistant and susceptible peach root stocks. J. Amer. Soc. Hort. Sci. 96:458-461.
- Kochba, J., and R. M. Samish. 1972. Effect of growth inhibitors on root knot nematodes in peach roots. J. Amer. Soc. Hort. Sci. 97:178-180.
- Kuc, J. 1966. Resistance of plants to infectious agents. Ann. Rev. Microbiol. 20:337-370.
- Kutsky, R. J., and T. E. Rawlins. 1950. Inhibition of virus multiplication by naphthalene acetic acid in tobacco tissue cultures as revealed by spectrophotometric method. J. Bacteriol. 60:763-766.
- Little, T. M., and F. J. Hills. 1972. Statistical Methods in Agricultural Research. U. C. Davis Book Store, Davis, California, 242p.
- McClure, M. A., T. H. Kruk, and I. Misaghi. 1973. A method of obtaining quantities of clean Meloidogyne eggs. J. Nematol. 5:230.
- McClure, M. A., and J. Robertson. 1973. Infection of cotton seedlings by M. incognita and a method of producing uniformly infected root segments. Nematologica 19:428-434.

- Mjuge, S. G., and D. R. Viglierchio. 1975. Short Communication. Influence of growth promoters and inhibitors on tomato plants infected with Meloidogyne incognita and M. hapla. Nematologica 21:476-477.
- Nelmes, A. J., D. L. Trudgill, and D. C. M. Corbett. 1973. Chemotherapy in the study of plant parasitic nematodes, pp. 95-112 in A. E. R. Taylor and R. Muller, eds. Chemotherapeutic agents in the study of parasites. Blackwell Scientific Publications, Oxford, England.
- Nusbaum, C. J. 1958. The response of root-knot infected tobacco plants to foliar application of maleic hydrazide. Abstr. Phytopathology 48:344.
- Orion, D., and G. Minz. 1969. The effect of Ethrel (2-chloroethane phosphonic acid) on the pathogenicity of the root-knot nematode Meloidogyne javanica. Nematologica 15:608-614.
- Orion, D., and G. Minz. 1971. The influence of morphactin on the root-knot nematode Meloidogyne javanica and its galls. Nematologica 17:107-112.
- Peacock, F. C. 1959. The development of a technique for studying the host parasite relationship of the root-knot nematode Meloidogyne incognita under controlled conditions. Nematologica 4:43-55.
- Peacock, F. C. 1960. Inhibition of root knot development on tomato by systemic compounds. Nematologica 5:219-227.
- Peacock, F. C. 1963. Systemic inhibition of root-knot eelworm (Meloidogyne incognita) on tomato. Nematologica 9:581-583.
- Peacock, F. C. 1966. Nematode control by plant chemotherapy. Nematologica 12:70-86.
- Peacock, F. C. 1968. The outlook for plant chemotherapy. 1st Congress of Plant Pathology, London. Abstr. p. 147.
- Reilly, J. J., and W. L. Klarma. 1972. The soybean phytoalexin, hydroxyphaseolin, induced by fungicides. Phytopathology 62:1113-1115.

- Richards, R. R. 1949. Responses of representative fungi to certain growth regulating substances. *Bot. Gaz.* 110:523-550.
- Romney, R. K., J. L. Anderson, and G. D. Griffin. 1974. Effects of DCPA on seedling infection by root-knot nematode. *Weed Sci.* 22:51-54.
- Rowell, J. B. 1949. The pathogenecity of *Alternaria dauci* f. *solani*. PhD thesis, Univ. Minn.
- Sawhney, R., and J. M. Webster. 1975. The role of plant growth hormones in determining the resistance of tomato plants to the root-knot nematode, *Meloidogyne incognita*. *Nematologica* 21:95-103.
- Smith, B. D., and A. T. K. Corke. 1966. Effect of (2-Chloroethyl)trimethylammonium Chloride on the Eriophyal Gall Mite *Cecidophyopsis ribis* Nal. and three fungus diseases of black currant. *Nature* 212:643-644.
- Steel, R. G., and J. H. Torrie. 1960. Principles and Procedures of Statistics. McGraw-Hill Book Company, New York. pp. 99-115.
- Strang, R. H., and R. L. Rogers. 1971. A microradioautographic study of ¹⁴C-Trifluralin absorption. *Weed Sci.* 19:363-369.
- van Andel, O. M. 1968. Shifts in disease resistance induced by growth regulators. *Neth. J. Plant Pathol.* 74 (suppl):113-120.
- van Emden, E. F. 1964. Effect of (2 chloroethyl)trimethyl ammonium chloride on the rate of increase of the cabbage aphid (*Brevicoryne brassicae* (L)). *Nature* 201:946-948.
- Veech, J. A. 1977. A possible new approach to the chemical control of nematodes. *J. Nematol.* 9: 184-185.
- Veech, J. A., and B. Y. Endo. 1970. Comparative morphology and enzyme histochemistry in root knot resistant and susceptible soybeans. *Phytopathology* 60:896-902.
- Webster, J. M. 1967. Some effects of 3,4-dichlorophenoxy-acetic acid herbicides on nematode infested cereals. *Plant Pathol.* 16:23.

